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14. ABSTRACT

The goal of this project is to exploit the potential of using fluorescence and diffuse reflectance spectroscopy for breast cancer detection during a core needle breast biopsy. A novel side-firing fiber optic probe has been developed for use in a vacuum-assisted core biopsy needle. The probe design has been evaluated using tissue phantom studies before embarking on the clinical study, and proved to be capable of making fluorescence measurements with good signal-to-noise ratio. Clinical trials have been carried out to use the optical probe for in vivo fluorescence spectroscopy of breast tissues during a core needle breast biopsy, to determine the feasibility of using this technique for a near real time discrimination between malignant and benign breast tissues. Preliminary results showed that in vivo fluorescence spectroscopy during a percutaneous breast biopsy is feasible and has the potential to quickly characterize tissue composition and pathology.

15. SUBJECT TERMS
Optic Probe, Fluorescence, Spectroscopy, Breast Cancer, Diagnosis, Biopsy

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INTRODUCTION

The long-term goal of this research is to develop an optical sensor based on tissue fluorescence and diffuse reflectance spectroscopy in ultraviolet-visible (UV-VIS) spectral range, which can potentially be used as an adjunct diagnostic tool to the core needle breast biopsy, and improve the sampling accuracy of this minimally-invasive procedure for breast cancer diagnosis. So far as we know, there has been limited work on *in vivo* fluorescence spectroscopy of breast tissues during breast biopsy procedures. The objective of this project is then to develop a prototype optical sensor (including fiber optic probe, instrument hardware and algorithm), and test the feasibility of *in vivo* optical spectroscopy of breast tissues during a percutaneous core needle biopsy and its potential for distinguishing between malignant and benign breast tissues.

BODY

Fluorescence and diffuse reflectance spectra can be measured using fiber optic probes, which is an important hardware component for tissue spectroscopy. The first task of this study has been to design and construct a fiber optic probe that can be incorporated into a currently used core needle for breast biopsy. A first generation probe has been designed for use in a 9-gauge vacuum assisted biopsy needle (Suros ATEC, Suros Surgical Systems, IN), which is currently employed for breast biopsy at the University of Wisconsin Health and Clinics (UWHC). The side-facing aperture at the tip of this biopsy needle provides a window through which light can be transmitted to and from surrounding breast tissues. The first generation probe consists of a centrally positioned fiber that delivers illumination light from the source to tissue, and two adjacent fibers that deliver the light emitted from tissue to the detector. The tips of these fibers were obliquely polished at an angle of 42° such that the light path are perpendicular to the fiber axis, to enable the illumination and collection of lights through the side-facing aperture of the needle. A patent application to protect the probe design has been submitted by the Wisconsin Alumni Research Foundation (WARF) [1]. The constructed fiber optic probe was quantitatively assessed using tissue phantom study before embarking on the clinical study. The phantom studies showed that this probe is capable of measuring fluorescence spectra with good signal-to-noise ratio (SNR). However the SNR for diffuse reflectance measurement needs improvement due to significant internal reflection in the probe.

A primary focus of my work in the past year was to use the side-firing fiber optic probe to measure breast tissue fluorescence and diffuse reflectance, *in vivo*, from patients undergoing a core needle biopsy procedure. The clinical protocol for the *in vivo* optical spectroscopy during core needle biopsy has been reviewed and approved by the University of Wisconsin's Institutional Review Board (IRB), and informed written consent was obtained from each enrolled patients. To date, we have successfully performed optical spectroscopy in 60 patients undergoing image-guided percutaneous breast biopsy, and a total of 100 samples were obtained for further analysis.

Preliminary data analysis was carried out on measurements from 82 (including 18 malignant and 64 benign) specimens with accompanying histological diagnosis. A set of spectral variables which represent fluorescence contribution from endogenous fluorophores present in breast tissues such as tryptophan, collagen, NADH (reduced Nicotinamide Adenine Dinucleotide), and ß-carotene, were extracted from the fluorescence measurements and evaluated for their correlation with subject age and tissue composition. Results from the analysis showed that the fluorescence intensity of collagen (indicating fibroglandular tissues) decreases, while that of ß-carotene (indicating adipose tissues) increases with subject age and percent adipose content in biopsy samples. This agrees with the fact that breast density decreases and adipose tissue content increases in older women [2]. The intensity ratio of tryptophan and collagen fluorescence and the intensity ratio of NADH and ß-carotene fluorescence displayed statistically significant differences between malignant, fibrous, and adipose tissues. These results suggested that *in vivo* fluorescence spectroscopy during a clinical breast biopsy procedure is feasible and has the potential to characterized tissue composition and pathology.

Results from the preliminary analysis for differentiation between malignant and benign breast tissues are not yet conclusive, as more malignant samples will be needed in order to systematically identify the spectroscopic features that show statistically most significant contrast between malignant and benign breast tissues. Given the fact that 70% - 80% of breast biopsies in clinic are benign (according to the American Cancer

Society) [3], a larger number of clinical trials are therefore needed to fully demonstrate the potential of this technique for *in vivo* breast cancer diagnosis. This work is currently underway, and we expect to continue to be able to consent four to five study subjects per month, which will yield an accrual of 50 ~ 60 participants in a course of one year. The next step in the data processing will be to carry out multivariate statistical analysis and classification analysis in order to distinguish malignant from benign breast tissues, when tissue specimens of statistically sufficient sample size are obtained.

KEY RESEARCH ACCOMPLISHMENTS

- ❑ Designed and constructed a novel fiber optic probe that is compatible with a currently used core needle for breast biopsy

- ❑ Quantitatively evaluated the probe design for fluorescence and diffuse reflectance measurements

- ❑ Established study protocol and logistics of clinical trials

- ❑ Carried out clinical trials and demonstrated the feasibility of *in vivo* optical spectroscopy of breast tissues during a clinical core needle biopsy procedure

- ❑ Carried out preliminary data analysis, evaluated the correlation of extracted spectral variables with subject age and tissue composition, and examined the spectral variables that show statistically significant differences between different tissue types

REPORTABLE OUTCOMES

- ❑ **Patent Application Publication**

Changfang Zhu, Carmalyn Lubawy, Nirmala Ramanujam, P03279US, "Side-firing probe for performing optical spectroscopy during core needle biopsy" September 2005 (Patent pending)

- ❑ **Conference Publication**

Changfang Zhu, Elizabeth S. Burnside, Gale A. Sisney, Josephine M. Harter, Nirmala Ramanujam, "In vivo Fluorescence Spectroscopy during Breast Core Needle Biopsy" Optical Society of America: Biomedical Topical Meeting, Fort Lauderdale, FL, March 2006.

CONCLUSIONS

Optical spectroscopy has the potential to be used as a diagnostic tool for breast cancer. Clinical trials are being carried out and have demonstrated the feasibility of *in vivo* optical spectroscopy of breast tissues during a clinical core needle biopsy procedure. Preliminary analysis of breast tissue fluorescence showed that fluorescence spectroscopy can be used to characterize tissue composition, thus has the potential to provide a near real time feedback to identify the optical sites for sampling during a core needle biopsy. If this optical technique proves to be diagnostically useful, it can set a precedent for an optically guided breast biopsy and potentially improve the diagnostic efficacy of breast biopsy at low cost.

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http://www.mayoclinic.com/health/mammogram/WO00023

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In vivo Fluorescence Spectroscopy during Breast Core Needle Biopsy

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Abstract: We carried out clinical trials where we measured breast tissue fluorescence spectra during core needle biopsy. This study demonstrated the feasibility of *in vivo* fluorescence spectroscopy for assessing breast tissue composition during a clinical biopsy procedure.
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OCIS codes: (170.4580) Optical diagnostics for medicine; (170.6510) Spectroscopy, tissue diagnostics; (300.6280) Spectroscopy, fluorescence and Luminescence; (300.6540) Spectroscopy, ultraviolet; (300.6550) Spectroscopy, visible

1. Introduction

Current core needle biopsy for breast cancer diagnosis has limited sampling accuracy. Incorporating an optical sensor into the biopsy needle to identify tissue types at the needle tip prior to biopsy could potentially improve its sampling accuracy and reduce the large number of unnecessary biopsies obtained for diagnosis. Our goal is to develop a prototype optical sensor based on ultraviolet-visible (UV-VIS) fluorescence spectroscopy and demonstrate its feasibility for breast cancer diagnosis during core needle biopsy. In this study, we have conducted clinical trials to measure fluorescence spectra of breast tissues, *in vivo*, during a core needle biopsy procedure, and results from the study suggest the potential of this technique for assessing breast tissue composition *in vivo*.

2. Methods

2.1 Probe design

A fiber optic probe has been designed for use in a vacuum-assisted core biopsy needle (Suros ATEC, Suros Surgical Systems, IN), which is currently employed for image guided breast biopsy at the University of Wisconsin Health and Clinics (UWHC). The 9-gauge Suros needle has a side-facing aperture that is 20 mm long and 3.7 mm wide at the needle tip. A side-firing probe has been configured to enable the illumination and collection of light through this aperture. The probe consists of a centrally positioned illumination fiber with a diameter of 600 μm and a numerical aperture (NA) of 0.22, and two surrounding collection fibers, each with a diameter of 200 μm and an NA of 0.22. The tips of these fibers are obliquely polished at an angle of 43° and radially oriented such that the light paths are perpendicular to the fiber axis. The fibers are aligned such that the center-to-center distance between the illumination and collection areas is approximately 600 μm at the probe surface. All the fibers are encased in a transparent quartz cap that transmits light in the UV-VIS spectrum and these fibers relay the optical signals to a spectrometer via flexible tubing.

2.2 In vivo optical spectroscopy during core needle biopsy

In vivo optical spectroscopy was carried out on patients undergoing either a stereotactic or an ultrasound guided core needle biopsy. During the biopsy procedure, the needle was positioned into the lesion with mammographic (stereotactic) or ultrasound guidance. The sterile probe was then inserted into the needle to make fluorescence measurements on breast tissues at the needle tip through the side-facing aperture. After the optical measurements the probe were retracted and a biopsy was taken from the site where measurements had been made on. The needle was then rotated to a new clock position. Optical measurements were made at a total of 6 clock positions within a 360-degree rotation. A biopsy was obtained from each corresponding site. The incorporation of optical spectroscopy extended the conventional biopsy procedure by approximately 15 – 20 minutes.

All measurements were made within UV-VIS spectrum using a multi-wavelength optical spectrometer. At each clock position, fluorescence emission spectra were recorded at nine excitation wavelengths from 300 to 460 nm in 20 nm increments. For each excitation wavelength, fluorescence emission spectra were measured over a 260 nm wavelength range, with the first wavelength shifted by 20 nm from the excitation wavelength.

2.3 Histopathology of breast samples

Each biopsy sample was submitted for histopathologic diagnosis, and the tissue composition was recorded as %adipose, %fibroglandular, %ducts/lobules, %benign, and %cancer. Benign tissues were further categorized as adenosis, fibroadenoma, cyst,

fibrocystic change and reparative changes, and cancerous tissues were divided into infiltrating ductal carcinomas and ductal carcinoma in situ. The samples were broadly classified as normal, benign and malignant based on the histologic composition.

2.4 Data analysis

The measured fluorescence spectra were calibrated to correct for (1) background fluorescence, (2) the wavelength dependent response of the system, and (3) the throughput of the system. Prior to spectral analysis, each calibrated spectrum was normalized to its integrated intensity in order to remove inter-patient variations and possible intra-patient variations due to variations in probe-tissue contact.

A set of spectral variables was extracted from the normalized fluorescence spectra. This included the fluorescence intensities at excitation-emission wavelengths of (300,340), (340,390), (360,460), and (340,520) nm, which correspond to fluorescence excitation-emission maxima of tryptophan, collagen, NADH and Vitamin A, respectively [1]. In addition, the ratios of fluorescence intensities at pairs of peak emission wavelengths at a given excitation wavelength of interest were also calculated. For convenience, the excitation-emission data point of the normalized spectra is denoted as $F(?_{exc},?_{emm})$, and the ratio of fluorescence intensities at two emission wavelengths is denoted as $F(?_{exc},?_{emm1})/F(?_{exc},?_{emm2})$.

For normal tissue samples, the set of spectral variables were examined for their variation with %adipose and age. A Spearman rank correlation [2] was employed to calculate the correlation coefficient. For all tissue samples, a Wilcoxon rank-sum test [2] was carried out on each of the spectral variables, in order to identify which ones show statistically the most significant differences between malignant and non-malignant breast tissues.

3. Results and Discussion

A total of 40 samples collected from 15 patients, including 7 malignant, 1 dominant benign, 14 dominant fibrous, 14 dominant adipose and 4 mixture of fibrous and adipose tissues, were analyzed in this study. Fig.1 shows the average excitation-emission matrices (EEMs) of (a) normal fibrous (n=14), (b) normal adipose (n=14) and (c) malignant (n=7) breast tissues. Three peaks are visible at similar locations in all three EEMs, i.e. at excitation-emission wavelength pairs of (300, 340), (340, 390), (360, 460) nm, which are attributed to tryptophan, collagen, and NADH, respectively [1,3]. The fluorescence peak at 360nm excitation in the normal adipose EEM extends from 460 to 520 nm. This emission peak may represent the fluorescence of Vitamin A [1], whose precursor is beta-carotene, which is primarily stored in adipose tissue [4]. Spearman correlation test indicated that this peak was highly correlated with the %adipose content in the breast samples (p < 0.01).
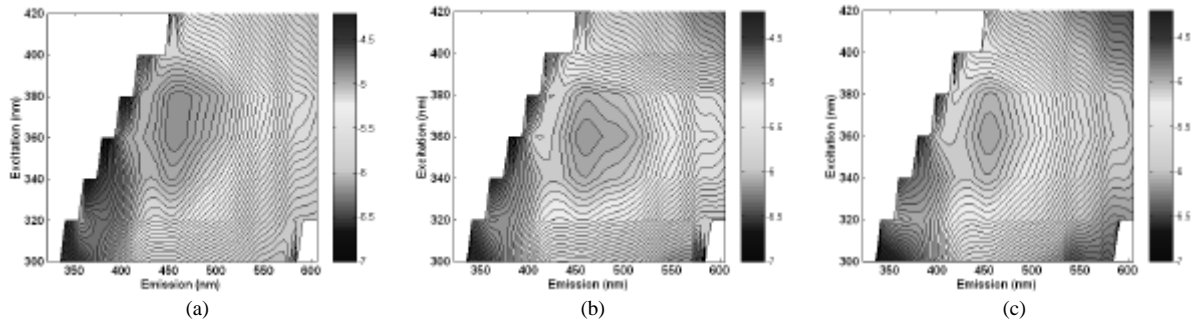

Fig. 1. Average EEMs of (a) normal fibrous (n=14), (b) normal adipose (n=14), and (c) malignant (n=7) breast tissues

Fig.2 shows (a) the average fluorescence spectra of the three tissue types at 320 nm, excitation, and (b) the ratio of fluorescence intensities $F(320,390)/F(320,520)$ as a function of patient age. Normal fibrous tissue displayed higher fluorescence at the emission wavelength of 390 nm, indicating that they are rich in collagen, while normal adipose tissues displayed relative higher fluorescence at 520 nm. The fluorescence intensity ratio $F(320,390)/F(320,520)$ decreased with patient age (p < 0.01). This agrees with the fact that the breast density decreases and the adipose tissue content increases in older women [5].
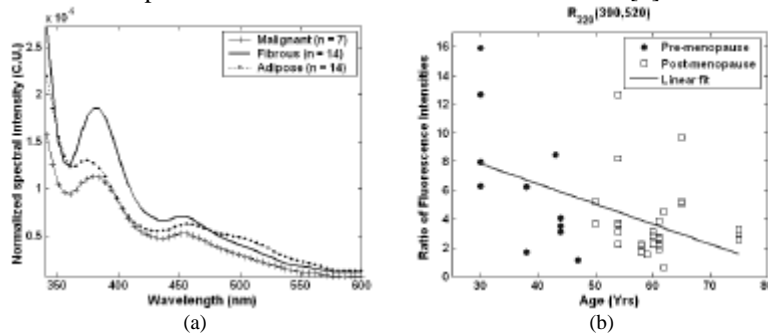

Fig. 2. (a) Average fluorescence spectra of the three tissue types at 320 nm, excitation, (b) the ratio of fluorescence intensities $F(320,390)/F$ (320,520) as a function of patient age

Table 1 lists the spectral variables identified from a Wilcoxon rank-sum test that show statistically the most significant differences between malignant and non-malignant breast tissues. Some of these spectral parameters are correlated, however, the scatter plot (Fig.4) of the two least correlated parameters, i.e. the normalized fluorescence intensity at (360, 460) nm and the normalized fluorescence intensity at (380, 520) nm, shows differences in the distribution of these parameters between malignant and non-malignant breasts.

Table 1. Spectral variables that show statistically the most significant differences between malignant and non-malignant breast tissues

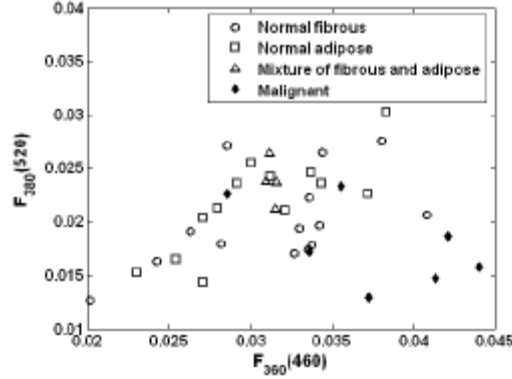| Spectral Variables	P-value
Normalized fluorescence intensity $F(360, 460)$	$p < 0.01$
Fluorescence intensity ratio $F(360, 460)/F(360, 520)$	$p < 0.02$
Fluorescence intensity ratio $F(380, 460)/F(380, 520)$	$p < 0.02$
Normalized fluorescence intensity $F(380, 460)$	$p < 0.05$
Normalized fluorescence intensity $F(380, 520)$	$p < 0.05$
Fluorescence intensity ratio $F(340, 460)/F(340, 520)$	$p < 0.05$

Fig. 3. Scatter plot of normalized fluorescence intensities at (360,460) and (380,520) nm.

This study demonstrates the feasibility of carrying out *in vivo* fluorescence spectroscopy of breast during a clinical core biopsy procedure. A larger number of clinical trials are need to fully demonstrate its potential for breast cancer diagnosis *in vivo*, and this work is currently underway.

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